REMARKS

Amendments to the Specification

The specification has been amended to provide a revised sequence listing. Specifically, a typographical error in SEQ ID NO:38 has been corrected. No new matter has been added. A diskette which contains the revised sequence listing in computer readable form as required by 37 C.F.R. § 1.821(e) is enclosed herewith, along with a paper copy of the Sequence Listing and a statement that the contents of the paper copy and the computer readable copy of the Sequence Listing are the same as required by 37 C.F.R. § 1.821(f).

The specification has been further amended to remove embedded hyperlinks and to generically refer to registered trademarks. No new matter has been added.

Claim Amendments

Applicants note with appreciation that claims 51, 52 and 99-102 have been indicated allowable. Applicants also note with appreciation that the Examiner has searched and examined each of the antibody sequences specifically recited in the claims, as requested by Applicants in response to the previous restriction requirement.

Claims 1-24, 28-30, 32-34, 36-38 and 40-104 were pending. Claims 1, 12, 14, 16, 18, 90 and 91 have been canceled without prejudice. New claims 105-108 have been added. Claims 57-66, 68-89, and 91-98 are withdrawn from consideration. Claims 2, 5, 6-11, 13, 15, 17, 19, 23, 24, 29, 30, 33, 34, 37, 38, 40, 43, 46, 49, 53-55, 57-65, 67-71, 73, 88, 89, 92-96, 103, and 104 have been amended.

Specifically, claims 2, 5, 6-11, 13, 15, 17, 19, 40, 43, 46, 49, 53-55, 57-65, 67-71, 73, 88, 89, 92-96, 103, and 104 have been amended to depend from claim 51 or 52.

Claims 23, 24, 29, 30, 33, 34, 37, 38 have been amended to correct a typographical error.

New claims 105-108 are drawn to human anti-CD25 antibodies having a specific human heavy CDR3 sequence. Support for new claims 105-107 can be found throughout the application as originally filed.

The foregoing claim cancellations and amendments should in no way be construed as acquiescence to any of the Examiner's rejections and have been made solely to expedite prosecution. Applicants reserve the right to pursue the claims as previously pending in this or a separate application(s). No new matter has been added.

Rejection of Claims 12, 14, 16, 18, 19, and 56 Under 35 U.S.C. §112, First Paragraph

Claims 12, 14, 16, 18, 19, and 56 are rejected as failing to comply with the written description requirement. Applicants respectfully disagree. However, to expedite prosecution, claims 12, 14, 16, and 18 have been canceled without prejudice. Further, claims 19 and 56 have been amended to depend from claim 51 or 52 to which this rejection does not apply. Accordingly, this rejection has been obviated.

Rejection of Claims 7-24, 28-30, 32-34, 36-38, 40-50, 53, 54, 56, 67, 103 and 104 Under 35 U.S.C. §112, First Paragraph

Claims 7-24, 28-30, 32-34, 36-38, 40-50, 53, 54, 56, 67, 103 and 104 are rejected as not being enabled. Applicants respectfully disagree. However, to expedite prosecution, claims 12, 14, 16, and 18 have been canceled without prejudice. Further, claims 2, 5, 6-11, 13, 15, 17, 19, 40, 43, 46, 49, 53-55, 57-63, 65, 67-71, 73, 88, 89, 94-96, 103, and 104 have been amended to depend from claim 51 or 52 to which this rejection does not apply. Accordingly, this rejection has been obviated with respect to these claims.

The remaining claims (claims 22-24, 28-30, 32-34, and 36-38) are drawn to fully human anti-CD25 antibodies defined by at least the heavy chain CDR3 sequence and conservative sequence modifications thereof. With respect to these claims, the Examiner maintains that "it is unlikely that human anti-CD25 monoclonal antibodies as defined by the claims, *i.e.*, modifications to the variable regions, and which may contain both framework and CDR substitutions for the heavy and light chain variable regions in unspecified order, have the required binding function." Applicants respectfully traverse this rejection for at least the following reasons:

It was well established at the time of filing that the CDR3 region alone could be used to generate antibodies having the same antigen specificity

As evidenced by the publications summarized in the enclosed Appendix A and attested to by Dr. Jan GJ van de Winkel in the enclosed Declaration, it was well established in the art at the time the present application was filed that the CDR3 domain alone can determine the binding specificity of an antibody and, importantly, that multiple antibodies can be predictably generated based on a common CDR3 sequence, without undue experimentation. Indeed, Applicants

further submit herewith for the Examiner's consideration a summary of recently issued US patents which include claims to antibodies defined by a single CDR sequence (Appendix H).

Specifically, the references summarized in Appendix A provide evidence supporting these art recognized aspects of antibody architecture and binding, and the role of the CDR3 region in particular. For example, Klimka *et al.* (enclosed as Appendix B) describe the production of a humanized anti-CD30 antibody (the same antigen bound by the claimed antibodies) using only the heavy chain variable CDR3 domain, *i.e.*, the major determinant for epitope-specificity, of a murine anti-CD30 antibody, Ki-4. The human version of the murine anti-CD30 antibody was produced by sequentially replacing the murine variable heavy and light chain genes with human V gene repertoires, while retaining only the heavy chain CDR3 domain of the murine Ki-4 antibody (see Abstract; page 253, column 1, second full paragraph; and page 255, "Results" section, last paragraph of column 1-column 2). As demonstrated by Klimka *et al.*, the anti-CD30 antibody was found to compete with the parental murine antibody for binding and to retain other functional characteristics of the parental murine antibody (*e.g.*, inhibits the shedding of the extracellular part of the CD30 receptor from L540 cells).

With regard to the importance of the retained VH-CDR3 domain, Klimka *et al.* explicitly state that this region is known "for its significant importance in determining the binding specificity of an antibody" (page 259, left column, first full paragraph). The authors also conclude that "this region was important in retaining the CD30-epitope specificity of the parental antibody in the humanized scFv." Still further, the authors note that the "importance of the VH-CDR3 for epitope specificity has also recently been reported by Beiboer *et al.* (2000), thereby confirming our findings" (page 269, left column, first full paragraph).

Like Klimka *et al.*, Beiboer *et al.* (enclosed as Appendix C) generated recombinant antibodies using only the heavy chain CDR3 sequence of a parent antibody. Specifically, the authors engineering an antibody to epithelial glycoprotein-2 (EGP-2) by retaining only the murine heavy chain CDR3 domain of the murine MOC-31 antibody. As confirmed in Beiboer *et al.*, "the heavy chain CDR3 is the main loop involved in antigen binding . . . " (page 839, left column, last full paragraph). The newly created antibody was found to bind the same epitope and have a similar binding affinity as the parental murine antibody.

Similarly, using only the CDR3 sequence of a parent antibody, Rader *et al.* (Appendix D) describe the production of a humanized anti-integrin $\alpha_v\beta_3$ antibody using the heavy and light

chain variable CDR3 domains of the murine anti-integrin $\alpha_v\beta_3$ antibody, LM609. Rader *et al.* report that several antibodies were produced having different sequences outside the CDR3 regions and capable of binding the same epitope as the parent murine antibody with affinities as high or higher than the parent murine antibody.

Further evidence showing that functionally equivalent recombinant antibodies could indeed be generated without undue experimentation at the time of the present invention using only the CDR3 sequence of a parent antibody and, importantly, the predictability of generating multiple antibodies having the same binding specificity based on a common CDR3 sequence is provided by Barbas *et al.* (1994) (Appendix E) who describe a method for generating antibodies having high affinity for double-stranded DNA. In particular, Barbas *et al.* successfully generated isolated antibodies by antigen selection from synthetic libraries which utilized the same heavy chain with randomized CDR3 sequences. The authors concluded that the CDR3 provides the most significant contribution to antigen binding (page 2161, left column, second full paragraph).

Moreover, in a separate publication, Barbas *et al.* (1995) (Appendix F) also describe grafting the heavy chain CDR3 sequences of three Fabs, SI-1, SI-40 and SI-32, against human placental DNA onto the heavy chain of an anti-tetanus toxoid Fab, thereby, replacing the existing heavy chain CDR3. The results of these studies showed that grafted Fabs produced binding to DNA (page 2532, second paragraph, and the Abstract) and, thus, that the CDR3 alone conferred binding specificity.

Similarly, Ditzel *et al.* (Appendix G) also describe grafting studies which showed that a heavy chain CDR3 only can be transferred to the heavy chain of another antibody and retain the same binding specificity. Specifically, the heavy chain CDR3 sequence of the polyspecific Fab LNA3 was grafted onto the heavy chain of the monospecific IgG tetanus toxoid-binding Fab p313, thus, replacing the existing heavy chain CDR3 (paragraph bridging columns on page 740). The binding specificity of the LNA3 heavy chain CDR3-grafted Fab (LNA3/p313) was tested in an ELISA against a panel of exogenous and autoantigens (Figure 3). LNA3/p313 bound to the panel of antigens as did the original LNA3 Fab (page 742, second column, through page 744, first column and Figure 3e).

The foregoing pre-filing publications, as well as the other publications and patents listed in Appendices A-H, clearly establish that the skill in the art at the time of the present invention was such that, once provided with the CDR3 sequence of a given antibody, one of ordinary skill

of the art could have generated other antibodies having the same CDR3 region and binding specificity, yet having different CDR1, CDR2 and framework regions, without undue experimentation.

It was well within the skill of the art at the time of filing to have identified and made conservative amino acid substitutions within CDRs that do not remove antibody binding

Claims 22-24, 28-30, 32-34 and 36-38 are drawn to human antibodies having particular CDR sequences and conservative modifications thereof. Accordingly, the presently claimed conservative sequence modifications represent a limited, art-recognized group of amino acid modifications that retain antigen binding function and, as such, the claims recite a clear limitation on the number of amino acids that can be modified.

As described in detail below, and in the enclosed Declaration by Dr. van de Winkel, it was well within the ordinary skill in the art to identify and test amino acid residues within the CDR domains of a given antibody that are amenable to conservative modifications, *i.e.*, conservative modifications which do not abolish antigen binding. Moreover, the identification of such conservative modifications would not have required undue experimentation and, in fact, involved routine techniques, such as those described in the present specification (see, *e.g.*, Example 3, pages 59-61, as well the references summarized in Appendix I).

For example, Brummell *et al.* ((1993) Biochem. 32:1180-1187; enclosed as Appendix J) used site-directed mutagenesis to examine the binding site of antibodies specific for *Salmonella*. Specifically, the CDR3 heavy chain domain was selected for study and a total of ninety (90) mutants were produced and screened by affinity electrophoresis / Western blots. Those of particular interest were further characterized by enzyme immunoassay and thermodynamic characterization by titration microcalorimetry. Brummell *et al.* found that antigen binding "was retained in a wide range of mutants with only one residue, Gly^{102H}, being irreplaceable."

Similarly, Kobayashi *et al.* ((1999) Protein Eng. 12(10):879-884), (enclosed as Appendix K), describes further methods to determine binding abilities of various mutant antibodies and states that "conservative substitution of Trp H33 by Tyr or Phe resulted in [only] moderate losses of binding affinity; however, replacement by Ala [*i.e.*, a non-conservative modification] had a significantly larger impact." (Abstract, comments in square brackets added).

Burks *et al.* ((1997) PNAS USA 94:412-417; enclosed as Appendix L) used PCR mutagenesis with *in vitro* transcription/translation and ELISA for the rapid generation and characterization of antibody mutants. Specifically, the authors analyzed the role and plasticity of six key contact residues in the binding pocket of a single chain Fv antibody derived from the anti-digoxin 26-10 murine antibody. A total of 114 mutant antibodies were produced. Approximately 75% of the single amino acid mutants exhibited significant binding to one or more of the digoxin analogs, even though non-conservative sequence modifications were permitted.

Other methods known in the art at the time of filing for identifying residues critical for antibody binding included, for example, comparing the antibody heavy and light chain variable region sequences to their respective germline sequences to identify which residues were amenable to conservative modification and which were not, *i.e.*, which residues had been conserved and which had been somatically mutated to improve binding.

Accordingly, the foregoing publications and enclosed Declaration clearly evidence the fact that the knowledge and high level of skill in the antibody art at the filing date of the present application were such that one of ordinary skill could have predictably identified and made conservative sequence substitutions which do not remove antibody binding, without undue experimentation, within antibody variable region and CDR sequences, such as those presently claimed.

The references relied on by the Examiner do not establish unpredictability with respect to generating fully human antibodies that bind to CD25 based on a common CDR3 sequence as presently claimed

The Examiner relies on several references in support of the rejection of claims 22-24, 28-30, 32-34, and 36-38 as not being enabled. Contrary to the Examiner's assertions, however, these references do not establish unpredictability or that undue experimentation was required to arrive at the claimed invention at the time of filing.

Specifically, the Examiner cites Burgess *et al.* and Schwartz *et al.* However, these references examine the effects of nonconservative modifications within particular molecules, instead of conservative modifications as required by the present claims. Specifically, these references describe replacing a lysine residue with glutamic acid and replacing a histidine residue with aspartic acid. Thus, both of these substitutions involved replacing an amino acid

containing a basic side chain with an amino acid containing an acidic side chain, *i.e.*, non-conservative substitutions.

The Examiner also cites Lin *et al.*. However, this reference fails to teach any substitutions whatsoever, and instead examines the effect of removing the amino terminal histidine of glucagons from an antibody CDR region.

The Examiner further relies on Lazar *et al.* which describe a conservative modification (replacement of aspartic acid with glutamic acid) that results in a reduction of binding activity. Accordingly, Lazar *et al.* (as well as the foregoing references of Burgess *et al.*, Schwartz *et al.*, and Lin *et al.*) in fact support enablement by showing that it was within the ordinary skill in the art at the time of the invention to have identified particular amino acid residues that are critical for binding.

Applicants acknowledge that certain amino acid changes in the variable regions of antibodies do, indeed, affect binding. However, the relevant issue is whether it would have required undue experimentation to identify such residues. While it may have been unpredictable to identify which exact residues within the relatively short CDR or variable region sequences of a given antibody that can be conservatively substituted, the important point is that it did not require undue experimentation at the time of filing to have done the necessary testing to identify these residues. Indeed, the references cited by both the Examiner and by Applicants clearly show this.

The Examiner also relies on Queen *et al.* and Rudikoff *et al.* to support the present enablement rejection. However, these references relate to the modification of humanized antibodies. Specifically, the teachings of Queen *et al.* and Rudikoff *et al.* pertain to CDR-grafted humanized antibodies. Unlike fully human antibodies, humanized antibodies include CDRs from a non-human antibody (*e.g.*, a mouse antibody) and a framework region from a human antibody. This requires modifications to the framework regions to maintain binding, because of the differences in antibody structure and incompatibilities between the species.

In contrast, the claimed fully human antibodies include CDRs from the same species and, thus, are inherently more stable (*e.g.*, by virtue of naturally occurring CDR-framework interactions) than humanized antibodies. In addition, as a result to their greater stability, fully human antibodies are more amenable to amino acid sequence modifications, particularly conservative modifications which do not affect charge, compared to other antibodies, such as humanized antibodies.

Accordingly, in the art of generating recombinant antibodies based on CDR regions (*e.g.*, a given CDR3) from another (parent) antibody, the predictability of maintaining binding of the parent antibody is far greater in the field of fully human antibodies *versus* humanized antibodies. For at least this reason, Queen *et al.* and Rudikoff *et al.* do not establish unpredictability with respect to generating fully human antibodies that bind to human CDC25 based on a common CDR3 sequence, as presently claimed.

Moreover, like Burgess *et al.* and Schwartz *et al.*, Rudikoff *et al.* describe studies in which non-conservative amino acid substitutions (*e.g.*, substitution of alanine for glutamic acid) were made within the variable heavy chain region, as opposed to conservative substitutions as presently claimed.

Again, the question whether certain amino acid modifications can affect binding of an antibody is not the relevant inquiry in the present case. The relevant inquiry is whether one of ordinary skill at the time of the present invention would have been able to make and use recombinant fully human antibodies which bind to human CD25 having at least the recited CDR3 sequence without undue experimentation. As discussed in detail above and evidenced by the enclosed supporting publications, the answer is clearly yes: that the claimed antibodies are fully enabled given the knowledge, high level of skill and predictability in the art at the time of the present invention, combined with the detailed teachings of Applicants' specification.

Rejection of Claims 1-6 and 55Under 35 U.S.C. §103(a)

Claims 1-6 and 55 are rejected as being unpatentable over Green (J. Immunol. Methods 231:11-23 (1999)) in view of Osterberg *et al.* (Biochem. Soc. Trans. 23:1038-1043 (1995)). Applicants respectfully traverse this rejection. However, to expedite prosecution, claim 1 has been canceled without prejudice. Moreover, claims 2-6 and 55 have been amended to depend from claim 51 or 52, to which this rejection does not apply. Accordingly, this rejection is now moot.

CONCLUSION

If a telephone conversation with Applicants' attorney would help expedite the prosecution of the above-identified application, the Examiner is urged to call the undersigned at (617) 227-7400.

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Respectfully submitted,

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